How Proteins Cross the Bacterial Cytoplasmic Membrane

Driessen, A.J.M.

Published in:
The Journal of Membrane Biology

DOI:
10.1007/BF00234937

Publication date:
1994

J. Membrane Biol. 142, 145–159 (1994)

The Journal of
Membrane
Biology

Topical Review

How Proteins Cross the Bacterial Cytoplasmic Membrane

A.J.M. Driessen
Department of Microbiology, University of Groningen, 9751 NN Haren, The Netherlands

Received: 22 March 1994/Revised: 23 May 1994

Introduction

The study of protein translocation across the bacterial cytoplasmic membrane is a rapidly advancing area of current biological research. In *Escherichia coli,* proteins are exported to the periplasmic space and outer membrane. These proteins are synthesized in the cytosol as precursors, mostly with a cleavable signal sequence. The pioneering work of Beckwith, Silhavy, Ito, and coworkers [for reviews, *see* refs. 17, 63, and 132; for a chronological perspective of important findings in bacterial protein export, *see* ref. 162] has established that the targeting and translocation of precursor proteins across the cytoplasmic membrane requires the products of at least six *sec*-genes. These genes, *secA, secB, secD, secE, secF,* and *secY,* encode interacting core-components of a complex translocation apparatus that consists of soluble, peripheral and membrane integrated proteins (Table, and Fig. 1) [For review, *see* 162]. Homologous genes have been found in many other Bacteria and even in Archaea, indicating that this pathway is widely distributed. In addition, several other factors have been found that may contribute to the translocation reaction. Sec proteins have been purified to homogeneity and reconstituted into liposomes [25]. These proteoliposomes translocate precursor proteins across the lipid bilayer in an energy-dependent manner. The mechanism of protein translocation across the bacterial cytoplasmic membrane and the membrane of the endoplasmic reticulum (ER) or thylakoid of eukaryotic cells may share many features in common [52, 57, 75]. It has been known for some time that signal sequences of these systems are functionally interchangeable. It has just recently become apparent that these systems also share homologous components, suggesting that protein translocation may occur via a common mechanism. In this review, I will concentrate on the biochemical role of the individual components of this general protein export pathway, and not discuss Sec-independent translocation or the alternative and dedicated protein translocation pathways that also exist in Bacteria [121, 153].

Protein Translocation Is Mediated by a Multisubunit *Translocase* Complex

In *E. coli,* precursor proteins are first recognized in the cytosol and maintained in a translocation-competent state by the SecB protein. SecB promotes the interaction of precursor proteins with the SecA protein. SecA is an ATPase, and at the membrane surface it interacts with acidic phospholipids and a multisubunit integral membrane protein complex consisting of the SecY, SecE and Band 1 or SecG[1] polypeptides. Together these proteins form a complex termed the *translocase. Translocase* mediates the ATP- and Δp-dependent translocation of precursor proteins across the membrane. SecD, and SecF are needed for the late stages of translocation. During translocation, the signal peptide is removed from the precursor protein by the membrane-bound signal peptidase SP-I or SP-II. The latter signal peptidase is specific

Key Words: Energetics — Membrane protein — Assembly — Folding — Transport

[1] For simplicity, I use the term SecY/E complex to indicate the integral membrane protein complex of SecY, SecE and "band 1." The latter is identical to component "P12" [39a]. *See* section on integral membrane components.

Table 1. Components implicated in protein export in *E. coli*

Component	Function	Molecular mass[a] (kD)	Location[b]	Copies per cell	Eukaryotic homologue
Translocase					
SecA	Translocation-ATPase, SecB-precursor protein receptor, molecular chaperone	102 (2)	im, c	2500–5000[c]	—
SecY	Channel subunit, signal sequence receptor	49	im	300–600[c,d]	SEC61αp
SecE	Channel subunit, stabilizes SecY	14	im	200–400[c,d]	SEC61γp
SecG	Band 1—Copurifies/associates with SecY/E	14.7[e]	im	?	SEC61βp?
	P12—Stimulates *in vitro* translocation	11.4	im	?	
Others					
SecB	Molecular chaperone, protein targeting to SecA	14 (4–5)	c	?	—
SecD	Protein release in periplasm, maintenance Δp	67	im	450–900[c]/7–30[d]	—
SecF	Maintenance Δp	35	im	30–60[c]/7–30[d]	—
Signal recognition particle					
Ffs	4.5 S RNA, SRP subunit		c	?	7 SL RNA
Ffh	Signal sequence binding factor, GTPase, SRP subunit	48	c	?	SRP54
FtsY	Subunit SRP receptor, GTPase	48	im, c	?	SRPα
Signal peptidases					
LepB	Signal peptidase I	36	im	?	SEC11p
LspA	Signal peptidase II	18	im	?	—

[a] Subunit composition indicated in parentheses; [b]im, inner membrane; cy, cytosol; p, periplasm; [c]from Ref. [99] and [d]Ref. [118]; [e]SDS-PAGE estimate; —/?, unknown.

for lipoproteins. Other proteins are required for proper folding, assembly in multisubunit structures and/or covalent modification with lipids. This highly efficient system handles protein translocation rates of up to 1,000 amino acid residues per sec, allowing an *E. coli* to double every 20 min.

SecB Is a Chaperone Specific to Protein Export

In *E. coli,* precursor protein synthesis and membrane translocation are not strictly coupled [123]. This led to the view that translocation of proteins across the cytoplasmic membrane is not driven by the ribosome's polypeptide chain elongation machinery *per se.* Precursor protein can be translocated as a completed polypeptide chain [123]. In the cytosol, molecular chaperones prevent the stable folding of precursor proteins. SecB is a homotetrameric chaperone protein with a specific function in protein translocation [32, 78, 161]. It forms a stoichiometric complex with precursor proteins [87], but does not catalyze folding or unfolding nor does it interact with ATP. SecB, however, stabilizes the unfolded protein in a soluble state, and hence may retard folding sufficiently to bring about a productive interaction of the precursor protein with other Sec proteins in order to become translocated. This view is supported by in vitro studies that demonstrate that precursor proteins can spontaneously form a translocation-competent state when diluted into solution from denaturant, while they lose their translocation competence during prolonged incubation in the absence of SecB [21, 32, 87, 91]. SecB retards the folding of the precursor of maltose binding protein (pre-MBP) [126], and prevents the formation of insoluble aggregates of the rapidly folding precursors of the outer membrane proteins OmpA [86] and PhoE [21]. SecB associates with the mature domain of precursor proteins [126], but the specificity of this binding reaction has remained elusive. Some precursor proteins, such as pre-ribose binding protein (pre-RBP) do not require

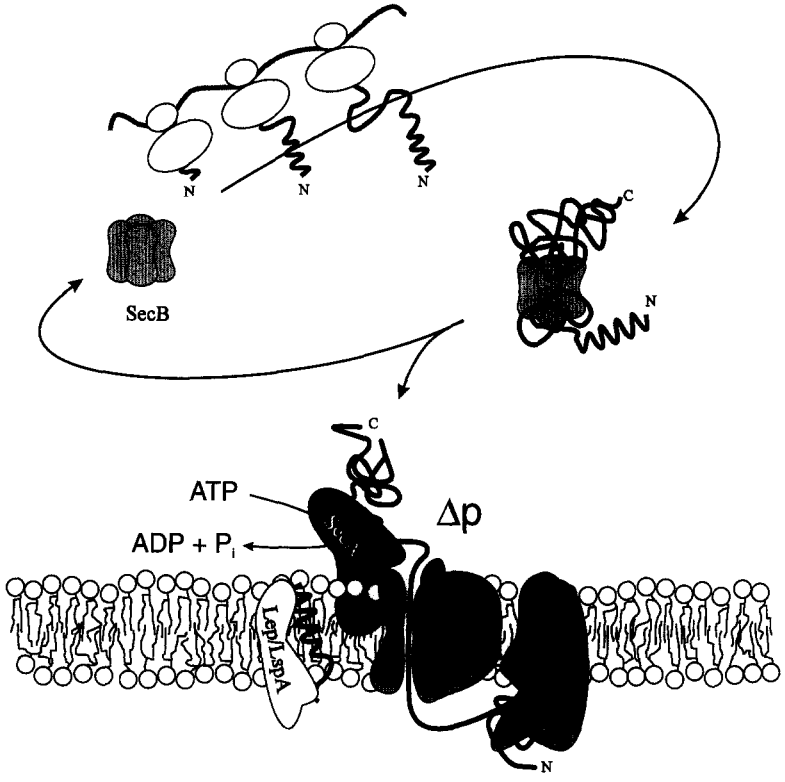

Fig. 1. Schematic overview of the Sec components involved in the general protein export pathway in *E. coli.*

SecB for export, but single point mutations that presumably affect the folding of pre-RBP, suffice to render its translocation SecB-dependent. It has been suggested that SecB recognizes the polypeptide backbone, and that it binds preferentially at sites exhibiting β-conformation. OmpA and PhoE bear a high amount of β-structure both in their final conformations and in the SecB bound precursor forms [21, 86]. SecB will also bind with low affinity to short peptides carrying a net positive charge (i.e., arginine and lysine residues) [124], including synthetic signal peptides (A.J.M. Driessen, *unpublished*). It has been suggested that the signal sequence domain of precursor proteins is essential for recognition by SecB [157]. Other studies suggest that the signal sequence facilitates binding of the precursor protein to SecB by retarding the folding of the mature domain [126]. In vitro data indicate that SecB will bind to a variety of unfolded proteins, but not to native proteins. The kinetic partition model proposes that SecB preferentially associates with slow folding proteins, while rapidly folding proteins are precluded from binding [55]. This model seems appropriate for the folding in the test tube, but it may not explain the high selectivity of SecB in vivo where it associates preferentially with polysome-bound nascent polypeptide chains of pre-MBP and a number of outer membrane proteins [79], while SecB is also needed for cotranslation translocation [80].

SecB is essential only in rapidly growing cells, suggesting that its function is required when the demand on the export pathway is high. The specific function of SecB in protein export may relate to its binding affinity for SecA that allows a rapid and specific targeting of bound precursor proteins to the membrane surface [31, 56]. SecB binds to SecA in solution, thereby promoting the binding of precursor proteins to SecA [56, A.J.M. Driessen, *unpublished*]. SecB may facilitate a functional interaction of the precursor protein with SecA by presenting the signal sequence in a proper conformation. This defines the role of SecB as a factor that binds to nascent polypeptide chains when they emerge from the ribosome, stabilizes the completely synthesized precursors in a translocation-competent conformation and directs them to the SecA subunit of the *translocase* in the cytosol.

Various other soluble chaperone proteins are involved in stabilizing newly synthesized precursor proteins. Induction of the heat-shock response substitutes for a defect in SecB function. This appears to be due to, as yet unidentified, heat-shock proteins other than GroEL/ES and DnaK [7]. These latter chaperones cannot replace SecB, and are involved in specific functions only [84, 85, 113].

Signal Recognition Particle: A Component of a Cotranslation Branch of the Sec Pathway?

Signal recognition particle (SRP) is essential for targeting of nascent chains to the ER membrane of Eukaryotes

[127]. It consists of one RNA (7 SL RNA) and six protein subunits. The N-terminal domain of the 54K subunit of SRP (SRP54) contains a GTP binding site, whereas the C-terminal domain binds signal sequences and SRP RNA. Binding of SRP to the signal sequence as it emerges from the ribosome creates a cytosolic targeting complex containing the nascent polypeptide chain, the translating ribosome, and SRP. This complex is targeted to the ER membrane and binds to the SRP receptor, a protein composed of two subunits, SRα and SRβ, both also containing a GTP-binding domain. SRP54 is stabilized by signal sequences, and binding of SRP to the SRP receptor activates SRP54 for GTP hydrolysis [102]. SRP then dissociates from both the signal sequence [107, 131] and the ribosome [51], that bind to SEC61p. SRP is released from the SRP receptor and recycled in the cytosol. The identification of a SRP homologue in *E. coli,* several other Bacteria and Archaea, suggests that this pathway is not confined to higher organisms [93]. The 4.5 S RNA of *E. coli* (the *ffs* gene product) has sequence homology to the 7 SL RNA of SRP, the *ffh* gene product, Fth, is homologous to SRP54, and the *ftsY* gene product is homologous to SRα [16, 120, 129]. While evidence indicates that the 4.5 S RNA of *E. coli* is involved in general protein synthesis [23, 53], it may also function in protein translocation as it associates with Fth into a ribonucleoprotein complex that interacts with the signal sequence part of nascent chains [95] and binds tightly to FtsY in a GTP-dependent manner [101]. The SRP secretion pathway in Eukaryotes utilizes a translational arrest mechanism, but there is no evidence that this occurs in *E. coli* [53]. The Fth protein, however, can substitute for SRP54 in a mixed reconstitution with eukaryotic SRP components with respect to the particle formation and the translation arrest activity [16]. This chimeric particle does not interact with the eukaryotic SRP receptor and fails to facilitate translocation activity. In vivo depletion or overexpression of 4.5 S RNA causes retardation of Bla export but not of other exported proteins [53, 119, 128]. Interestingly, Bla shows the slowest post-translational export kinetics in *E. coli,* and does not require SecB but uses GroEL [84, 85]. Depletion of Fth [114] or depletion or overexpression [94] of FtsY causes growth arrest and export retardation of several precursor proteins. In vitro, FtsY depletion retards the export of Bla approximately twofold [94]. FtsY localizes at or near to the cytoplasmic membrane in *E. coli* [94], suggesting that it may interact with other membrane components (*see below*), or perhaps an as yet unidentified homologue of the mammalian SRβ.

Although the precise relationship between the SRP pathway and the Sec pathway is not clear at present [13, 93], certain parallels exist between the bacterial and mammalian systems. The SRP pathway may function as an extension to the Sec pathway. For instance, it may fulfill an early chaperone role, and transfer nascent chains to SecB/SecA. Alternatively, it may permit cotranslational translocation at the *translocase* complex or an as yet unidentified *translocase* complex possibly consisting of FtsX, FtsH (*see* section on integral membrane components), and/or the FtsE protein that is homologous to the ATP-binding component of the ABC (ATP binding cassette) family of transport systems. In analogy with the mammalian SEC61p [51], SecY/E may bind ribosomes. This may be especially relevant to the membrane insertion of proteins. Due to their hydrophobicity these proteins are difficult to stabilize in the cytosol in a soluble form. There is, however, no clear consensus as to whether polytopic membrane proteins use the Sec pathway for membrane integration or not [1, 64]. Some membrane proteins seem to require SecY and not SecA [146], opening the exciting possibility that SecA can be bypassed and that the SecY/E protein complex can be directly accessed. The SRP and chaperone pathways may thus coexist for general protein export, converge at the SecY/E protein complex and/or differ in the timing of nascent chain association.

SecA Is the Dissociable, ATPase Subunit of the *Translocase*

SecA is a dissociable subunit of the *translocase* [162, 164]. Structural analysis [M. Spiering, A.J.M. Driessen, E. Boekema, *unpublished*] of the SecA protein bound to the lipid surface indicates that SecA exists as a (homo-)dimer [3]. The protein retains its dimeric structure during translocation, and studies on the "half-of-sites" activity demonstrates that the dimer is the catalytic unit [42]. SecA functions as a receptor for the SecB-precursor protein complex [56] and couples ATP hydrolysis to the translocation of precursor proteins across the membrane [89, 111]. Many of the functions of SecA are presently revealed and these are discussed in this section.

SecA Is a Precursor Protein-stimulated ATPase with Two ATP-binding Domains

SecA harbors a low endogenous ATPase activity that is enhanced up to 100-fold when it interacts with precursor proteins, the SecY/E protein complex, and anionic phospholipids [25, 89, 90]. This activated ATPase activity, i.e., "translocation ATPase," is needed for in vitro translocation. Azide is a specific inhibitor of the translocation ATPase [110], and blocks both in vitro and in vivo translocation. Many of the azide-resistant mutants of *E. coli* [110] and *Bacillus subtilis* [R. Freudl, *personal communication*] map in the *secA* gene. SecA is the only ATPase that is needed and sufficient to drive in vitro protein translocation [89]. The protein can be photoinactivated by the nucleotide-analogue 8-azido-ATP that binds to two distinct sites on the SecA protein. Inner membranes

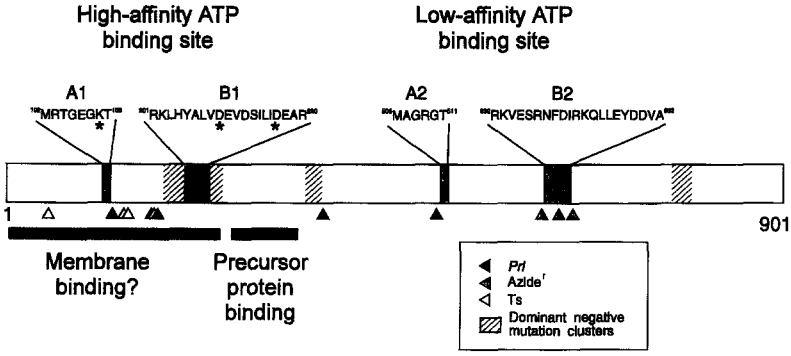

Fig. 2. Domain structure of the SecA protein. Regions that are possibly involved in membrane and precursor protein interactions are shown. Hatched areas indicate clusters of dominant-negative mutations [66], and the triangles indicate point mutations that render the protein temperature-sensitive (*Ts*), azide-resistant (*Azide*^{r}) or allow the translocation of precursor proteins with defective signal sequences (*Prl*). *A1* and *B1*, and *A2* and *B2* represent the regions with similarity to the Walker A and B motifs as found in NTP-binding proteins.

treated with 8-azido-ATP are inactive for translocation, and activity can be restored by adding back fresh SecA [89]. The two nucleotide-binding sites on SecA have been localized by cross-linking studies and analyzed by site-directed mutagenesis [100, 105, 146]. The amino-terminus harbors the high-affinity nucleotide-binding site ($K_{D,ADP}$ 0.13 μM) with similarity to the typical Walker A and B sequence motifs found in many ATP-binding proteins (Fig. 2; *A1* and *B1*) [156]. Mutations in the critical residues of both domains have a strong effect on translocation and SecA translocation ATPase activity [74, 105, 164]. The region in SecA corresponding to the B-domain is rather atypical as it shows an adjacent duplication of this motif, i.e. (i) one with homology to the DEAD box found in RNA-binding proteins, such as helicases [77], and (ii) one that aligns with the B domain of the ATP-binding site of ABC proteins. The latter is well conserved among SecA proteins. Both domains, however, are important for SecA function and both are involved in Mg^{2+}- and ATP hydrolysis [165; J. van der Wolk, M. Klose, R. Freudl, A.J.M. Driessen, *unpublished*]. The first region may be more specific for the ATP-dependent interaction of SecA with its own messenger RNA which is important for autogenous regulation of translation [39, 138].

The second nucleotide-binding site of SecA is of low affinity ($K_{D,ADP}$ 300–500 μM) (Fig. 2; *A2* and *B2*) [105]. This region likely has a regulatory function only as in vitro, protein translocation can be driven by ATP concentrations as low as 1 μM, i.e., about two orders of magnitude lower than the K_D for the second domain. Many of the dominant-negative mutants in SecA cluster around the two ATP-binding motifs that may cooperatively interact [66, 105; J. van der Wolk, A.J.M. Driessen, *unpublished*].

SecA Forms A Soluble Ternary Complex with Precursor Proteins and SecB

SecA is present in the cell in an approximately 10-fold molar excess compared to the other components of the *translocase*. A large portion of the SecA is found in the soluble fraction of a cell lysate and some SecA appears to be associated with ribosomes [88]. Membrane-associated SecA binds SecB with high affinity (K_D 150–250 nM), and both proteins form an isolable binary complex in solution [56]. The cellular localization of SecB and SecA suggests that they act early in the protein transl(oc)ation cascade. Precursor proteins associate with SecA in solution, and SecB promotes this interaction by binding SecA [56] and preventing the premature release of precursor protein from SecA [A.J.M. Driessen, *unpublished*].

The in vitro analysis of the interaction of SecA with precursor proteins has provided detailed insight into the series of events that finally leads to the initiation of translocation. Several *PrlD* (*prl* stands for *p*rotein *l*ocalization) mutations, which suppress export defects caused by signal sequence defects, have been found in the *secA* gene [18, 133]. Biochemical evidence demonstrates that SecA recognizes precursor proteins by binding both the signal sequence and mature domain [4, 34, 73, 90]. A typical signal sequence consists of a positively charged amino-terminus, a central hydrophobic region and a hydrophilic region containing the signal peptidase cleavage site [58]. Precursor proteins with a larger number of positively charged residues at the amino-terminus bind with higher affinity to SecA and translocate more efficiently in vitro [73]. Precursor proteins bind to SecA at a position adjacent to the high-affinity ATP-binding site [73]. Synthetic signal peptides known to inhibit translocation [29] compete with precursor proteins for binding to SecA [34]. High levels of ATP antagonize this inhibitory effect [34]. Conversely, soluble SecA releases bound ADP when it binds precursor proteins [140] and this event stimulates ADP-ATP exchange at the SecA protein. Synthetic signal peptides have a similar effect, but the stimulated ATPase activity is seen only when the signal peptide is added in conjunction with the mature domain [90]. Soluble SecA binds precursor proteins with higher affinity when liganded with ADP, and this binding reaction provokes a conformational change in the SecA protein [140]. ATP hydrolysis drives the release of the pre-

cursor protein from SecA [136]. Consistent with this notion, a mutation in the ATP-binding site of SecA that prevents the hydrolysis of ATP converts the protein into a state where it binds tightly to precursor proteins [164].

These observations can be summarized in the following model: (i) the signal sequence domain of a precursor protein binds to SecA, (ii) the SecA-bound ADP is discharged, (iii) SecA changes conformation and binds the mature region of the precursor protein, (iv) ATP binds to SecA, and (v) dislocates the bound signal sequence domain. Finally, (vi) ATP is hydrolyzed, and (vii) the mature domain is released from SecA. In solution, the turnover of SecA is very slow, allowing cycles of precursor protein release and rebinding. SecB promotes the SecA-bound state of the precursor protein. At the membrane, SecA is activated for ATP hydrolysis and this drives the release of the precursor protein which in turn enters the translocation pathway.

SecA Insertion into the Membrane May Drive Translocation

About 10–40% of the cellular SecA associates with the membrane [26]. SecA-phospholipid interactions are thought to be essential for protein translocation. Cells depleted of acidic phospholipids are severely blocked in protein translocation [155]. These results are obscured by the pleiotropic effect of acidic phospholipid depletion on the energetic state of the cells [154]. In vitro experiments established that acidic phospholipids are required for membrane binding [59] and translocation ATPase activity of SecA [90]. The translocation defect of inner membranes or reconstituted proteoliposomes depleted from acidic phospholipids can be restored by the reintroduction of these lipids [82]. The amount of SecA associated with the cytoplasmic membrane or liposomes also increases with increasing acidic phospholipid content of the membrane [83]. The interaction of SecA with the lipid surface is presumably electrostatic and of poor affinity. The unabated interaction of SecA with anionic phospholipids renders the protein thermolabile [90]. ATP, translocation-competent precursor proteins [90], and the SecY/E protein complex [25] stabilize the lipid-bound SecA. This process is termed "SecA lipid-ATPase," and is suppressed by divalent cations such as Mg^{2+}. Purified SecA penetrates efficiently into a lipid monolayer containing acidic phospholipids [20]. ATP hydrolysis prevents this insertion and favors a soluble state of the SecA protein, whereas it does not prevent membrane insertion of a SecA ATPase mutant [164]. In the ADP-bound state, SecA associates with the membrane surface, while in the presence of nonhydrolyzable ATP analogues the inserted state dominates. At the lipid surface, SecA unfolds [122] into an inactive, aggregated state [A.J.M. Driessen, *unpublished*].

How do these findings on the insertion of SecA into artificial membranes relate to the in vivo function of SecA? With liposomes, SecA will only bind with low affinity to the membrane [59]. The presence of the integral components of the *translocase* allows high-affinity binding (K_D 40 nM) [56]. SecA bound to these high-affinity sites exposes domains to lipid [122]. In the presence of a nonhydrolyzable ATP analogue SecA can temporally span the entire membrane exposing a region to the periplasmic face of the membrane [44a]. In summary, these data suggest that SecA may undergo nucleotide-modulated cycles of membrane insertion and exclusion [20]. Coinsertion of the bound precursor protein with SecA may allow the translocation of small polypeptide domains across the membrane as outlined in Fig. 3. SecA is only slowly released from the high-affinity binding sites, but in the presence of ATP it is rapidly replaced by free SecA [164]. This process is blocked in a SecA ATPase mutant [164], further supporting the notion that the membrane-associated state of SecA is modulated by its interaction with nucleotides. SecA may act in *trans* such that once it has completed the initiation of translocation, it is replaced at the translocation site by cytosolic SecA loaded with a newly synthesized precursor protein.

An Evolutionary Conserved Complex of Membrane Proteins That Constitutes the Transmembrane Translocation Pathway

Most of the genes identified as components required for protein export code for integral membrane proteins [132]. The membrane-integrated domain of the *translocase* consists of three polypeptides, i.e., SecY, SecE and a protein termed "band 1" (or SecG, *see below*) [25]. SecY and SecE are essentially for the viability of *E. coli,* and are needed for in vitro and in vivo protein translocation [63, 162]. SecY is a polytopic membrane protein predicted to span the membrane ten times [63]. SecE contains three transmembrane segments (TMS) [134]. Only the carboxyl-terminal TMS plus attached sequences suffice for activity [133]. In many other Bacteria, SecE harbors only a single TMS showing homology to the carboxyl-terminal region of the *E. coli* SecE [57, 106]. Band 1 is a protein that copurifies and coimmunoprecipitates with SecY and SecE as a three-component complex [25] that is labile at ambient temperature [24]. This complex (SecY/E complex) has been purified to homogeneity by virtue of its ability to support SecA translocation ATPase [25, 43]. In reconstituted form, it mediates multiple turnovers of SecA-dependent precursor-protein translocation and is as active for protein translocation as inner membrane vesicles [15, 25]. SecY and SecE can also be isolated separately from overproducing cells and can then be reconstituted together to yield active proteoliposomes [2, 108, 151]. The activity of these proteoliposomes, however, is low and a new

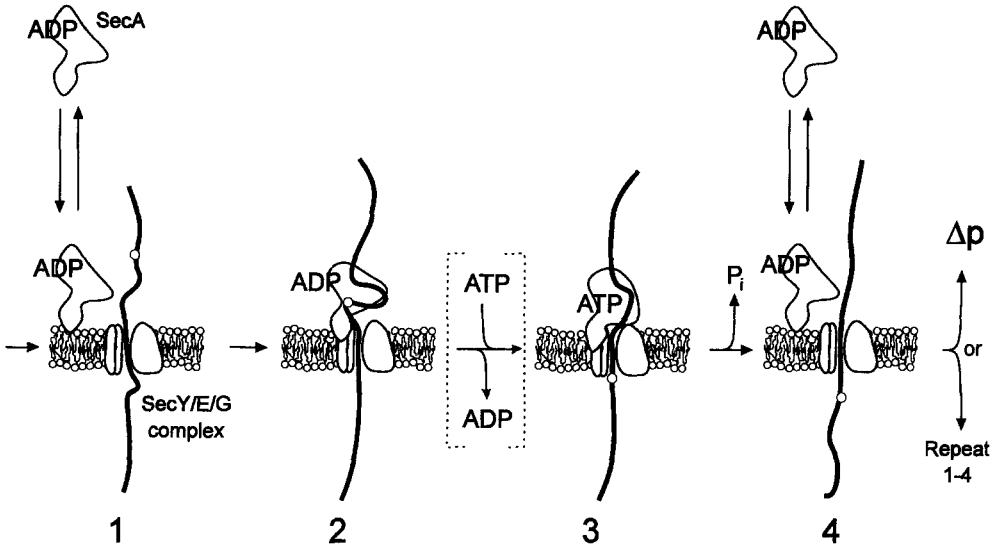

Fig. 3. Sewing model for the intermediate stages of protein translocation. SecA with bound ADP associates with the membrane surface and binds with high affinity to a polypeptide domain of a membrane-spanning translocation intermediate that is trapped by the SecY/E/G complex (step 1). This binding event promotes the translocation of a small polypeptide domain of the intermediate (step 2), and the interaction of SecA with the precursor protein and the SecY/E/G complex activates it for ADP-ATP exchange. Binding of ATP facilitates the insertion of SecA into the membrane or SecY/E/G complex, and allows further translocation of a small polypeptide domain of the intermediate through coinsertion (step 3). Subsequent hydrolysis of ATP by SecA drives the release of the bound intermediate and promotes the exclusion of SecA from the membrane-bound state (step 4). At that stage, translocation is further driven by Δp, or SecA can rebind the translocation intermediate to drive further limited translocation (repeat of steps 1–4). SecA with bound ADP is surface localized and can be replaced by cytosolic SecA.

membrane protein, P12, has been identified that dramatically enhances translocation [109]. P12 is a small membrane protein predicted to span the membrane twice. Disruption of the *p12* gene confers cold sensitivity to protein export [109a]. P12 and band 1 share many physicochemical properties in common. Recent evidence shows that both proteins are identical and form the third component termed SecG [39a]. The three-component organization of the *translocase* appears to be conserved in Bacteria, Archaea and Eukarya. Recently, the mammalian SEC61p complex of the ER has been purified and functionally reconstituted into proteoliposomes [52]. This complex also consists of three polypeptides, i.e., SEC61α and γ (also known as SSS1) which are homologous to SecY and SecE [51, 57], and SEC61β which may be the functional homologue of P12 or "band 1." The conserved nature of the molecular organization of the *translocase* complex strongly suggests that the basic mechanism of protein translocation in Bacteria, Archaea and the ER of Eukaryotes is similar.

Biochemical evidence indicates that SecE determines the stable existence of SecY in the cell [97, 150]. In wild-type cells, the newly synthesized SecY immediately associates with SecE and "band 1" to form a complex that does not dissociate during translocation [68a]. This contrasts with genetic data arguing that SecE and SecY are dissociatable subunits [18]. Mutations in SecY that affect the association between SecY and SecE are clustered in the second cytosolic loop (C4 domain) of SecY. The presence of *prl* mutations in the *secY* (*prlA*), and *secE* (*prlG*) genes suggests that SecY and SecE interact with the signal sequence domain. Most conditional lethal mutations in *secY* and *secE* are cold sensitive. Temperature-sensitive mutations are known only for *secA* and *secY*. Pogliano and Beckwith [117] proposed that the protein translocation pathway includes some intrinsic cold-sensitive steps, and a lowering of the activity in a step following the cold-sensitive step invariably leads to the cold-sensitive export phenotypes. A potential cold-sensitive step is the insertion of the signal sequence into the membrane before it may associate with the SecY/E complex.

Several factors have been identified that stabilize overexpressed SecY and suppress the dominant negative phenotype of *secY*$^{-d}$*1*, a gene that codes for an inactive SecY protein with an internal deletion [K. Ito, *personal communication*]. When expressed from a multicopy plasmid, SecY^{-d}1 sequesters SecE and competes with wild-type SecY for the formation of functional translocation complexes [139]. One of these suppressors is Ydr, a small hydrophilic protein that is able to partially stabilize the wild-type SecY. The function of Ydr is unclear as the disruption of its gene has no effect on protein translocation. Ydr does not functionally replace SecE, but may only mimic SecE by stabilizing SecY. The amino-terminus of Ydr bears homology to the conserved cytosolic region of SecE [T. Shimoike, A.J.M. Driessen, K. Ito, *unpublished*]. Likewise, expression of *yajC*, a gene upstream in an operon that contains the *secD* and *secF* genes [118], from a high-copy plasmid stabilizes

overexpressed SecY [K. Ito, *personal communication*]. *yajC* codes for a small membrane protein, but there is no evidence that it represents another *sec* gene [118].

It has been suggested that SecY is not required for translocation [160]. The SecY protein is not soluble in the detergent cholate, and this property can be used to obtain reconstituted proteoliposomes depleted from SecY. These proteoliposomes contain an assembly of unspecified membrane proteins and translocate a carboxyl-truncated form of pre-MBP in a SecB- and SecA-dependent manner [159, 160]. This contrasts with the overwhelming genetic and biochemical data that SecY requires for translocation [*see* reviews 63, 132, 162] and are conserved in nature. The discrepancy between these observations is difficult to explain, but it does not seem to be appropriate to blame it on the methodology used to assay translocation without a critical assessment of the generated data [159] realizing that it concerns comparison of a crude [158] with a well-defined system [2, 15, 24, 25, 41, 108, 109, 151]. The folding properties of the truncated pre-MBP may render this protein less SecY dependent than native substrates like pre-MBP, pro-OmpA and pre-OmpF which translocate in the purified system. In this respect, it is important to emphasize that the *translocase* was purified based on its ability to activate SecA for precursor protein-stimulated ATPase activity [25], and that the purified complex contained components that were previously identified through extensive genetic screenings [17, 132].

The exact function of the membrane-embedded domain of the *translocase* is unknown. So far, only indirect evidence indicates that SecA may bind at a site near to SecY [45]. Antibodies directed against SecY interfere with high-affinity binding of SecA to membranes [56] and prevent translocation [158]. SecA protects SecY against proteolysis by externally added proteinase [56]. The SecY/E complex may act as a transporter or pore, allowing precursor proteins to traverse the membrane, either through its center or along its surface. Translocation intermediates, i.e., precursor proteins that are trapped in an intermediate stage of translocation, can be specifically photocross-linked to the SecA and SecY proteins [68]. Neither SecE nor band 1 are identified with this technique, while phospholipids are nearly completely protected from cross-linking. SecA and SecY thus physically interact with the translocating polypeptide chain. In this respect, the mammalian SEC61α has been specifically cross-linked to translocation intermediates that differed in the position of the photoactivatable cross-linker such that a complete turn of a putative α-helical segment of the intermediate was covered [105a]. These data strongly suggest that the translocation intermediate is surrounded by a proteinaceous wall formed by the SEC61p.

Does the SecY/E complex function as a protein-conducting pore? Black lipid membrane studies with everted inner membrane vesicles or spheroplasts of *E. coli* show that the addition of a synthetic signal peptide provokes a large increase in the conductivity of the membrane [143]. These observations have been taken to suggest that precursor proteins are translocated through a proteinaceous aqueous pore that opens upon binding of the signal sequence domain. There is no evidence for the involvement of Sec proteins in this process, nor is it clear whether these channels also translocate proteins. Furthermore, the fact that this phenomenon depends on a non-physiological polarity of the transmembrane potential ($\Delta\psi$), i.e., negative on the *trans*-side instead of positive [143], is unsatisfactorily discussed. It will be of interest to determine if this signal peptide-dependent ion channel is also present in SecY-depleted membrane vesicles [160]. *E. coli* inner membrane vesicles show an enhanced halide permeability when conditions are used that result in the accumulation of translocation intermediates [137]. Similar observations have been made for the proton permeability of membrane vesicles containing elevated levels of SecY and SecE protein [72]. This increased halide permeability is also observed with liposomes reconstituted with the SecY/E complex, and is suppressed by the SecA protein (i.e., like a cork on a bottle) [A.J.M. Driessen, *unpublished*]. The ion channel opens again when a translocation-intermediate is trapped in the *translocase*. Channel formation is not triggered by synthetic signal peptides. Rather, they prevent translocation at an early stage by competing with precursor proteins for SecA binding. Similar electrophysiological measurements have been carried out with microsomes derived from the mammalian ER [142]. When fused to a black lipid bilayer, large ion channels appear when the polysomes are dissociated from the nascent chains and the membrane surface. The permeability barrier between the cytoplasm and the ER lumen is thought to be maintained by a tight junction between the ribosome and the translocator, i.e., the SEC61p complex. Current evidence, both in the bacterial and eukaryotic systems, favors the idea that proteins are translocated across the membrane along a proteinaceous surface [67].

Is the SecY/E protein complex a passive component or truly involved in catalysis—for instance, in energy transduction, protein unfolding and/or recognition. Proteins bearing an internal nonpolypeptide stretch do translocate, indicating that peptide-backbone recognition is not required throughout the translocation reaction [70]. The SecY/E complex, however, must be able to recognize stop-transfer sequences (or TMS) in translocating membrane proteins to release these proteins into the cytoplasmic membrane. The reconstituted SEC61p complex mediates the insertion of class I and class II membrane proteins [52]. There are no data available on the bacterial system in this respect; however, additional components may be required for the assembly of these proteins. One possible candidate is FtsH, a membrane-bound ATPase that belongs to a family of proteins that include the Eukaryotic counterparts of the ER/golgi

(SEC18p, NSF), mitochondrion (MSP1, BCS1) and peroxisomes (PAS1p). FtsH depletion or mutations in the *ftsH* gene interferes with the assembly of membrane proteins and export of Bla and OmpA [5, 6]. FtsH depletion may interfere with the assembly of the *translocase* subunits, thereby indirectly causing an export defect. FtsH may be a component of the *translocase* that is involved in the recognition of stop-transfer signals in translocating proteins. It may mediate the opening of the *translocase* subunits to allow the lateral exit of membrane proteins into the lipid phase.

Anionic Phospholipids Are Needed Early in Translocation

Along with their role in targeting the precursor protein to SecA, positive charges at the amino-terminus of the signal sequence may play an additional role in the early stages of translocation. The positively charged amino-terminus of the signal sequence of precursor proteins may interact electrostatically with anionic phospholipids in the membrane, possibly favoring the insertion of the hydrophobic core into the bilayer via the formation of a loop with the mature amino-terminus [58, 62]. Much indirect evidence has been gathered to support this view. A tentative correlation exists between the ability of synthetic signal peptides to insert into model membranes and to act as efficient targeting signals in protein translocation [22]. The total hydrophobicity of the core region is an important determinant for signal sequence function [60]. For instance, the requirement for a positive charge at the amino-terminus can be compensated for by a longer central hydrophobic stretch [61]. Under those conditions, translocation becomes less dependent on anionic phospholipids [115], suggesting that precursor proteins with a "classic" signal sequence indeed encounter the anionic phospholipids at an early stage of the translocation reaction. Anionic lipids are also required for the initial Sec-independent insertion of M13 procoat [81], possibly reflecting a primordial route of translocation. In subsequent steps, the signal sequence may associate with the SecY/E complex. It is not known if lipids are functionally involved in the later stages of translocation. Signal sequence-phospholipid interactions are not considered in models describing the translocation of proteins into the mammalian ER. Rather, experimental evidence in the yeast ER indicates that during an early stage in translocation, the signal sequence of nascent chains is in close contact with SEC61p [107, 131], while recent data with dog pancreas microsomes suggest that the signal sequence passes through an aqueous compartment in the membrane during translocation possibly at the SEC61p [33].

A Model for the Catalytic Cycle of the *Translocase*

Translocation of proteins through the Sec system has been shown to be dependent on two different sources of free energy: ATP hydrolysis and the protonmotive force, Δp (Δp is composed of a transmembrane electrical potential, $\Delta\psi$, and the pH difference across the membrane, ΔpH) [27, 28, 37, 40, 44, 49, 167, 168]. This dual energy requirement may be unique for bacterial protein translocation. ATP (or GTP) is required for most protein translocation processes that occur across biological membranes. Generally, NTP hydrolysis drives the release of precursor proteins from their association with molecular chaperones. Protein import into mitochondria requires $\Delta\psi$ only for the initial electrophoretic movement of the signal sequence across the inner membrane [112]. Some proteins imported into the thylakoid of the chloroplast specifically utilize ΔpH for translocation and membrane insertion [30], while import of proteins into the chloroplast or the ER appears to be totally independent of a Δp [127]. Detailed in vitro studies with the bacterial system have led to the definition of a catalytic cycle of the *translocase*. This model proposes that translocation proceeds in a stepwise manner [136], and is discussed in the following paragraphs.

ATP Hydrolysis by SecA Is Needed to Initiate Translocation

At the membrane, the targeting cascade recommences with the binding of the SecA subunit to the integral components of the *translocase* [56]. Several scenarios can be envisioned based on the possible existence of a lipid surface-bound, freely diffusible intermediate composed of the ternary complex of SecA, SecB and the precursor protein [56]. Such a complex may laterally diffuse to the translocation site, and eventually replace a previously bound SecA subunit. So far, there is no evidence that a lipid-bound ternary complex is a true intermediate in the targeting cascade. This complex may as well directly associate with the SecY/E protein [56]. SecA uses the energy of ATP binding to insert into the membrane [20] and to release the signal sequence domain of the bound precursor protein [136]. The signal sequence domain may then interact with anionic phospholipids (*see previous section*), adopt a loop-like structure with the mature amino-terminal region and insert into the membrane or the translocation channel. In vitro studies have demonstrated that in the presence of non-hydrolyzable ATP analogues, precursor proteins are translocated across the membrane to the extent that the signal sequence can be cleaved by signal peptidase [136]. Since the active site of signal peptidase is exposed to the periplasmic face of the inner membrane, about 40–45 residues must have been translocated. The length of the hydrophobic core of the signal sequence domain (10–15 amino acid residues) varies considerably among precursor proteins and is too short to span the entire membrane in α-helical conformation [58]. Penetration of SecA into the lipid bilayer

may serve to shorten the distance for the amino-terminus to get across, or facilitate a stable interaction of the signal sequence with "putative" binding sites on the SecY/E complex. Initial anchoring at the SecY/E complex and subsequent binding of the mature domain may prevent precursor proteins from diffusing away from the translocation site. The interaction between SecA and the SecY/E protein complex activates SecA for ATP hydrolysis. This process drives the release of the precursor protein from SecA and may promote exlcusion of SecA from the membrane-inserted state [20, 164]. These series of events ensure that the precursor protein is released by SecA at the translocation site, and preclude an unproductive insertion of the precursor protein into the lipid bilayer as observed in model systems [136]. It is not clear at what stage SecB is released from the precursor protein. In vitro, SecB is already redundant when the ternary complex has docked at the membrane translocation site [56].

Δp Drives Translocation in the Absence of SecA

Once translocation has been initiated at the expense of ATP, further translocation may proceed stepwise through a series of transmembrane intermediates, each with distinct energy requirements. This process can be separated in SecA-dependent steps (which requires ATP), SecA-independent steps (driven by Δp), and steps most likely driven by interaction of the preprotein domain with the *translocase* and/or phospholipids [48, 136, 148]. In the intermediate stages of translocation, SecA can rebind a translocation intermediate, insert into the membrane and through coinsertion drive the forward membrane translocation of a 4 kD polypeptide stretch (Fig. 3) [136]. Binding of ATP may then promote further coinsertion of SecA with bound precursor protein into the membrane, allowing the limited translocation of another 4 kD stretch. Subsequent ATP hydrolysis elicits the release of the protein from its association with SecA and may facilitate membrane deinsertion of SecA. Multiple cycles of these SecA-dependent translocation steps ultimately lead to the complete translocation of the polypeptide chain [136]. This process is very inefficient and slow and requires the hydrolysis of numerous ATP molecules [40]. Truncated pro-OmpA derivatives require less ATP for translocation [14]. In vivo, it seems more likely that Δp drives further translocation once SecA has released the precursor protein to the SecY/E complex. Δp permits rapid and efficient translocation of intermediates provided that they are not associated with SecA [40, 136]. Δp-driven translocation is completely blocked when the precursor protein associates tightly with SecA, i.e., with nonhydrolyzable ATP analogues [136] or when SecA is unable to hydrolyze ATP through a mutation in the ATP-binding site [164]. Excess SecA suppresses the Δp-dependency of precursor-protein translocation [166] as it favors rapid rebinding of released precursor proteins. The observation that translocation is more dependent on Δp when SecA is limiting is consistent with this explanation [166]. The intermediate stages of translocation are readily reversible and SecA-mediated ATP hydrolysis is not strictly coupled to net precursor protein movement along the translocation path [136]. Futile cycles of ATP hydrolysis occur when translocation is prevented by a stable tertiary structure in the precursor protein [136, 149] or when a Δp of reversed polarity is imposed [40]. In the absence of SecA association, "backward" translocation of intermediates can take place [136] that is prevented by Δp [40]. These futile cycles are presumably the cause of poor coupling between translocation and ATP hydrolysis found in vitro. Phenomenologically, Δp increases the coupling ratio between ATP hydrolysis and precursor protein translocation [40]. Mechanistically, these processes are completely distinct [136].

In vivo, the main catalytic role of SecA may be to initiate translocation at the expense of ATP and to donate the precursor protein to the SecY/E protein complex during a transient interaction to allow further Δp-driven translocation [41, 136]. SecA may then be needed only occasionally at later stages of the translocation reaction to permit transit of stable-folded tertiary structures in the polypeptide chain [147, 149]. Alternatively, SecA may drive the complete translocation reaction in a stepwise fashion, while Δp prevents backward translocation and thus ensures unidirectionality (*see* section on Δp).

SecD and SecF Facilitate Translocation at a Late Stage

SecD and SecF are both membrane proteins that were identified by cold-sensitive mutations that cause the accumulation of precursors of exported proteins [46]. The number of SecD and SecF molecules in the cell is approximately 1/10 the number of SecY and SecE molecules [99, 134], although there is uncertainty on the precise abundance of SecD [99, 118]. None of the *prl* mutations mapped in the *secD* and *SecF* genes, and it has been suggested that they are not involved in signal sequence recognition [19, 132]. Both proteins have large periplasmic domains positioned between the first two of six TMS [116, 117], and are not required for the biochemically reconstituted translocation reaction [25]. *secD* and *secF* null mutants are viable only at high temperatures and show a severe, cold-sensitive export defect [118]. Overexpression of SecD and SecF improves the export of proteins with defective signal sequences [118]. Genetic evidence indicates that SecD and SecF are dissociable subunits of the *translocase* at a late stage of translocation [19]. They are not associated, however, with the isolable SecY/E complex. Protein export in spheroplasts is in-

hibited when incubated with an antibody against SecD [98] and the data suggest that SecD is needed or improves the efficiency of the release of the proteins in the periplasm. SecD and SecF are not required for clearing of the precursor protein from the translocation site *per se,* since the reconstituted *translocase* mediates multiple rounds of translocation in their absence [15]. Depletion of SecD and SecF severely affects the ability of cells to maintain Δp [9]. This may be the reason for the translocation defect in *secD* and *secF* mutants. In vitro studies demonstrate that SecD and SecF are not needed for ATP-dependent translocation, but that they kinetically affect the Δp-dependent portion of the translocation reaction [9]. SecD and SecF, however, are not essential for coupling of Δp to translocation as shown with the reconstituted system [25, 40]. Why are *secD* and *secF* null mutants leaky for protons? Since translocation is slow in these mutant strains, leakiness could possibly arise from *translocase* units stuffed with translocation intermediates (*see* section on SecY/E protein). These units would present a major energy sink in cells causing the dissipation of Δp. By rapidly clearing the *translocase,* SecD and SecF may prevent the occurrence of such undesired ion fluxes. In many aspects, the in vivo dissipation of Δp resembles the effect of the depletion of SecD and SecF. In the presence of an uncoupler, a membrane-bound processed intermediate of pre-MBP accumulates at the periplasmic side of the inner membrane [47]. Δp may thus be involved in the release of MBP, and possibly also other precursor proteins. Precursor proteins are affected in different ways in their translocation in SecD and SecF mutant strains. This effect may be related to their Δp dependency of translocation.

Several other factors have been found that affect folding and/or release of exported proteins on the external face of the membrane. Signal peptidases I and II are needed to remove the signal peptide domain of precursor proteins and prelipoproteins [35]. The periplasm of *E. coli* contains at least two functional homologues of the eukaryotic protein disulfide isomerases (PDI). DsbA [12, 69, 163] and DsbC [104, 135] are periplasmic proteins believed to be involved in the oxidation of cysteine residues and disulfide-bond rearrangements in exported proteins. DsbA, whose molecular structure has recently been solved [96], is a periplasmic protein that acts in concert with the membrane-bound DsbB [11]. It has been suggested that DsbB specifically reoxidizes DsbA via the electron transfer chain, thus enabling it to recycle [11, 103]. In the absence of DsbA, these proteins remain bound at the periplasmic membrane surface in a reduced and unfolded state. Homologues of the SecA, SecE and SecY polypeptides have been found in a wide variety of Bacteria. As yet unique to Gram-positive Bacteria are PrsA and PrtM, both small, membrane-anchored, lipoproteins that are involved in the maturation of exported proteins in *B. subtilis* and *Lactococcus lactis,* respectively [54, 65, 76]. These proteins may function as folding factors that act in concert with the Sec system.

What Forces Drive Proteins across the Membrane?

Do Proteins Translocate by Diffusion and Brownian Motion?

What forces drive proteins across the membrane, and does active unfolding take place? It is generally believed that precursor proteins need to be in a so-called "loosely folded state" in order to be translocated [125]. This term is not well defined as it does not indicate the degree of folding. Translocation-competent precursor proteins show significant elements of final secondary and tertiary structure [21, 86]. This translocation-competent conformation, however, does not resemble the "molten-globular," a conformation found in early intermediates in the protein-folding pathway which exhibits a native-like secondary structure and a collapsed tertiary structure lacking any stable elements. The *translocase* mediates the translocation of short segments with tertiary structure, i.e., a segment stabilized by a disulfide bridge, provided that both energy sources (ATP and Δp) are present [147, 149]. Since precursor proteins are translocated as partially extended polypeptide chains, the need for unfolding is evident. Protein unfolding during translocation can be driven by the translocation reaction itself as shown by studies with a fusion protein consisting of the cytosolic dihydrofolate reductase (Dhfr) linked to the carboxyl-terminus of pro-OmpA [8]. This fusion protein translocates up to the folded Dhfr-moiety when stabilized by NADPH and methotrexate. Removal of these ligands allows a two-stage translocation reaction, i.e., (i) a spontaneous translocation of 20–30 amino acid residues concomitant with unfolding of the Dhfr domain, and (ii) SecA and ATP-dependent completion of translocation. Transfer of the amino-terminal domain of Dhfr to an energetically more favorable position drives the unfolding of Dhfr [8]. The same principle may apply to the "backward" and "forward" translocation of unstable intermediates as, for instance, very late pro-OmpA intermediates that translocate to full length when freed from a synthetically imposed translocation arrest [136]. Earlier intermediates remain at their position in the absence of SecA association [136], and require rebinding by SecA and cycles of ATP binding and hydrolysis or Δp for further translocation. By binding the polypeptide chain, SecA may contribute to unfolding since this event will restrict the freedom of the bound polypeptide segment which may be sufficient to bring about local unfolding. Next, SecA drives translocation by "pushing" the protein into the channel, and by binding the protein it will prevent "backward" translocation. The association

of the polypeptide chain with the SecY/E protein complex (with or without phospholipids) may also contribute to unfolding, and allow translocation until a new, energetically favorable position is attained [8]. In addition, protein binding or folding factors and covalent modifications of the polypeptide chain in the periplasmic space may shift the equilibrium so as to allow further translocation. In many respects, this model resembles the "ratchet model" [144] that proposes that translocation through a proteinaceous pore is driven solely by diffusion and Brownian motion in polypeptides and that the proteinaceous components of the translocation apparatus and energetic parameters only serve to direct translocation. Does the "ratchet" model adequately describe protein translocation in the bacterial system? The answer must be no if protein translocation is driven by the ATP-dependent insertion of SecA into the membrane. Moreover, unfolded proteins do not translocate spontaneously in the bacterial system, rather continuous input of energy is required for translocation.

The Unresolved Mystery of Δp-driven Translocation

How does Δp function as a driving force in protein translocation? This major question in the study of protein translocation is still a mystery. In vivo and in vitro studies have demonstrated that $\Delta\psi$ and ΔpH are equivalent forces in translocation [10, 40]. This observation strongly argues in favor of an energy-coupling mechanism in which H^+s are directly involved. This is further substantiated by the observation that in the marine bacterium *Vibrio alginolyticus,* protein translocation may be coupled to the sodium motive force [152]. There is no direct evidence that protein translocation is truly coupled to H^+ movements. The in vivo data utilizing uncouplers are complicated by the fact that ΔpH may affect translocation in a dual manner, i.e., as a driving force and via alterations of the cytosolic pH. In vitro studies demonstrate that the activity of the *translocase* is adversely affected by a lowering of the pH at the cytosolic face of the membrane [44]. Nevertheless, other studies demonstrate that ΔpH acts as a true driving force, implying that H^+s are involved in the translocation mechanism. One possible mechanism would be an H^+/polypeptide antiport in which protein export is coupled to an inward H^+ flux [41]. Attempts to detect such vectorial H^+-transfer reaction have failed thus far. Detection of these fluxes is complicated by the appearance of H^+ leaks that accompany the in vitro translocation reaction when intermediates jam the channel [72, 148; A.J.M. Driessen, *unpublished*]. On the other hand, the $\Delta\psi$-driven chase of a translocation intermediate of pro-OmpA is retarded more than threefold in deuterium oxide relative to the rate in water [44]. Such a kinetic solvent isotope effect is indicative for critical H^+-transfer reactions in a rate-limiting step. These H^+-transfer reactions, however, do not necessarily reflect vectorial H^+-translocation steps.

Studies with translocation intermediates demonstrate that Δp drives translocation of large protein domains without ATP and SecA [136, 148], and small, stable-folded domains in the presence of ATP [147, 149]. The "putative" translocation channel may adopt a more relaxed conformation in the presence of Δp to allow the transmembrane translocation of large, folded domains. Δp would thus act by widening the size of the translocation channel to allow "ratcheting" of the translocating polypeptide [144]. A reversed Δp would restrict its size thereby preventing "forward" translocation. Such a model has to invoke a transmembrane H^+ flux to explain the requirement for the total Δp. Binding factors at the periplasmic face of the membrane are needed to prevent "backward" translocation of the polypeptide chain in the absence of SecA. Certain mutations in SecY allow the translocation of signal sequenceless proteins, and one may argue that this loss of specificity is due to a relaxation of the translocation channel [38]. It is not known if these mutations affect the Δp dependency of translocation.

Precursor proteins vary in their requirement for Δp for translocation [167]. This may be related to the presence or absence of stable-folded tertiary structure elements [147, 149], the number and affinity of SecA-binding sites in the polypeptide chain assuming that there is some specificity in these interactions, and the charge distribution along the polypeptide chain. Precursor proteins bearing a mature domain devoid of ionizable residues still require Δp for translocation [71]. This implies that Δp performs a mechanistic function rather than acting upon the precursor protein itself as, for instance, through protonation/deprotonation or electrophoresis. This latter process may be relevant to the initial insertion of the signal peptide domain into the membrane [36], and several studies indicate that the charge [50] or structure [92] of the mature amino-terminal region of pro-OmpA is an important factor for the Δp dependency of processing. Other observations indicate that Δp may modulate the activity of SecA by reducing the apparent K_m of the translocation reaction for ATP thereby allowing Δp-driven translocation at a low ATP concentration [141]. This phenomenon has been attributed to an accelerating effect of Δp on the rate of ADP release from SecA. An indirect modulating role of Δp seems more evident and may be found in the ability of Δp to clear the precursor protein from the translocation site once it has been released by SecA [41].

Δp is essential for translocation in vivo, whereas it is only stimulatory in vitro [41]. This apparent discrepancy may be the result of multiple phenomena, i.e., (i) the in vitro systems used so far to analyze protein translocation cannot attain the high levels of Δp found in respiring cells, (ii) release of translocated proteins into the periplasmic space requires Δp [47], and this step is usually not assayed in vitro, (iii) in vitro studies use an excess SecA that suppresses the Δp dependency of trans-

location [136, 159, 167], and (iv) the collapse of Δp in vivo also results in a lowering of the internal pH, which adversely affects the activity of the *translocase* [44].

Concluding Remarks

Now that the complicated process of protein translocation as it occurs in the cell can be reconstituted with purified components, major research questions can be addressed at a more detailed molecular level. These are: What is the molecular mechanism by which Δp drives translocation? For instance, a biochemical demonstration is needed to establish if the channel opening varies with Δp. Further comparison of the energetics of bacterial protein export and the ΔpH-dependent protein import into the thylakoid may reveal salient features of the mechanism of energization through Δp. Other important questions relate to how membrane proteins integrate and assemble into the cytoplasmic membrane, and which steps are Sec dependent and independent. The function of the small subunits of the *translocase* needs to be resolved. Do they, for instance, act as valves that control the opening and closing of the channel in the lateral direction to allow the release of membrane domains. Is the folding state of precursor proteins modulated by interaction with Sec proteins, and which features of the mature domain of precursor proteins besides the signal sequence domain are recognized by Sec proteins will be important to reveal. This information may promote the use of bacterial cells as production factories for the secretion of heterologous proteins that often are only poorly secreted. How is the process of the translocation of outer membrane proteins across the cytoplasmic membrane linked to their final assembly into the outer membrane? In Gram-positive Bacteria it will be of interest to determine to what extent the cell wall represents a bottleneck for the secretion of proteins [145]. Studies are needed on the physiological role of protein export in differentiation processes, such as the development of natural competence and sporulation in *Bacillus* species, or the possible reversal of protein translocation in *E. coli* upon invasion by the parasite *Bdellovibrio crescentus* [130]. We will learn more about the general features of protein export systems by studying the dedicated protein export routes in Bacteria involved in the secretion of specific proteins across the outer membrane, with or without the accumulation of periplasmic intermediates [121, 153]. Now that homologues of the major components of the bacterial protein translocation system have been found in Archaea and Eukarya, in particular mammals and yeast, the protein translocation research field will converge. Major questions are: What is the role of the SRP pathway in *E. coli* and other Bacteria? How do these components interact with the *translocase?* What is the role of FtsH in this process? Does the SRP pathway function as an extension or alternative route to the Sec pathway? How are these pathways evolved in Archaea? In addition to the well-established powerful genetic and biochemical tools, biophysical experimentation will add further detail to our understanding of the molecular mechanism of protein translocation. A stage has been reached where detailed information on the structure of the *translocase* is urgently needed.

The author thanks Koreaki Ito, Bill Wickner, Hajime Tokuda, Kunio Yamane, Tom Rapoport, Jan Tomassen, Chris Murphy, Colin Robinson, Joen Luirink, Jan-Maarten van Dijl, and Roland Freudl for discussion and allowing the citation of their work prior to publication, and the members of his laboratory, especially Tanneke den Blaauwen and Peter Fekkes for critical reading of the manuscript. This work was supported by grants from the Netherlands Organization for Scientific Research (N.W.O.), the Netherlands Foundation for Chemical Research (S.O.N.), and the European Community (E.E.C.).

References

1. Ahrem, B., Hoffschulte, H.K., Müller, M. 1989. *J. Cell Biol.* **108:**1637–1646
2. Akimaru, K., Matsuyama, S.-I., Tokuda, H., Mizushima, S. 1991. *Proc. Natl. Acad. Sci. USA* **88:**6545–6549
3. Akita, M., Sasaki, S., Matsuyama, S.I., Mizushima, S. 1989. *J. Biol. Chem.* **265:**8164–8169
4. Akita, M., Shinkai, A., Matsuyama, S.-I., Mizushima, S. 1991. *Biochem. Biophys. Res. Commun.* **174:**211–216
5. Akiyama, Y., Ogura, T., Ito, K. 1994. *J. Biol. Chem.* **269:**5218–5224
6. Akiyama, Y., Shirai, Y., Ito, K. 1994. *J. Biol. Chem.* **269:**5225–5229
7. Altman, E., Kumamoto, C. A., Emr, S. 1991. *EMBO J.* **10:**239–245
8. Arkowitz, R. A., Joly, J. C., Wickner, W. 1993. *EMBO J.* **12:**243–253
9. Arkowitz, R. A., Wickner, W. 1994. *EMBO J.* **13:**954–963
10. Bakker, E.P., Randall, L.L. 1984. *EMBO J.* **3:**895–900
11. Bardwell, J.C.A., Lee, J.-O., Jander, G., Martin, N., Belin, D., Beckwith, J. 1993. *Proc. Natl. Acad. Sci. USA* **90:**1038–1042
12. Bardwell, J.C.A., McGovern, K., Beckwith, J. 1991. *Cell* **67:**581–589
13. Bassford, P., Beckwith, J., Ito, K., Kumamoto, C., Mizushima, S., Oliver, D., Randall, L., Silhavy, T., Tai, P.C., Wickner, W. 1991. *Cell* **65:**367–368
14. Bassilana, M., Arkowitz, R.A., Wickner, W. 1992. *J. Biol. Chem.* **267:**25246–25250
15. Bassilana, M., Wickner, W. 1993. *Biochemistry* **32:**2626–2630
16. Bernstein, H.D., Poritz, M.A., Strub, K., Hoben, P.J., Brenner, S., Walter, P. 1989. *Nature* **340:**482–486
17. Bieker, K.L., Phillips, G.J., Silhavy, T. 1990. *J. Bioenerg. Biomembr.* **22:**291–310
18. Bieker, K.L., Silhavy, T.J. 1990. *Cell* **61:**833–842
19. Bieker-Brady, K.L., Silhavy, T.J. 1992. *EMBO J.* **11:**3165–3174
20. Breukink, E.J., van Demel, R., de Korte-Kool, G., de Kruijff, B. 1992. *Biochemistry* **31:**1119–1124
21. Breukink, E.J., Kusters, R., de Kruijff, B. 1992. *Eur. J. Biochem.* **208:**419–425
22. Briggs, M.S., Gierash, L.M. 1986. *Adv. Prot. Chem.* **38:**109–180
23. Brown, S. 1989. *J. Mol. Biol.* **209:**79–90
24. Brundage, L., Fimmel, C.J., Mizushima, S., Wickner, W. 1992. *J. Biol. Chem.* **267:**4166–4170
25. Brundage, L., Hendrick, J.P., Schiebel, E., Driessen, A.J.M., Wickner, W. 1990. *Cell* **62:**649–657

26. Cabelli, R.J., Dolan, K.M., Qian, L., Oliver, D.B. 1991. *J. Biol. Chem.* **266**:24420–24427
27. Chen, L., Tai, P.C. 1985. *Proc. Natl. Acad. Sci. USA* **82**:4384–4388
28. Chen, L., Tai, P.C. 1986. *J. Bacteriol.* **168**:828–832
29. Chen, L., Tai, P.C., Briggs, M.S., Gierasch, L.M. 1987. *J. Biol. Chem.* **262**:1427–1429
30. Cline, K., Ettinger, W.F., Theg, S.M. 1992. *J. Biol. Chem.* **267**:2688–2696
31. de Cock, H., Tommassen, J. 1992. *Mol. Microbiol.* **6**:599–604
32. Collier, D.N., Bankaitis, V.A., Weiss, J.B., Bassford, P.J., Jr. 1988. *Cell* **53**:273–283
33. Crowley, K.S., Reinhart, G.D., Johnson, A.E. 1993. *Cell* **73**:1101–1115
34. Cunningham, K., Wickner, W. 1989. *Proc. Natl. Acad. Sci. USA* **86**:8630–8634
35. Dalbey, R.E., von Heijne, G. 1992. *Trends Biochem. Sci.* **17**:474–478
36. Daniels, C.J., Bole, D.G., Quay, S.C., Oxender, D.L. 1981. *Proc. Natl. Acad. Sci. USA* **78**:5396–5400
37. Date, T., Goodman, J.M., Wickner, W.T. 1980. *Proc. Natl. Acad. Sci. USA* **77**:4669–4673
38. Derman, A.I., Puziss, J.W., Bassford, P.J., Beckwith, J. 1993. *EMBO J.* **3**:879–888
39. Dolan, K.M., Oliver, D.B. 1991. *J. Biol. Chem.* **266**:23329–23333

39a. Douville, K., Leonard, M., Brundage, L., Nishiyama, K.-L., Tokuda, H., Mizushima, S., Wickner, W. 1994. *J. Biol. Chem.* **269**:18705–18707

40. Driessen, A.J.M. 1992. *EMBO J.* **11**:847–853
41. Driessen, A.J.M. 1992. *Trends Biochem. Sci.* **17**:219–223
42. Driessen, A.J.M. 1993. *Biochemistry* **32**:13190–13197
43. Driessen, A.J.M., Wickner, W. 1990. *Proc. Natl. Acad. Sci. USA* **87**:3107–3111
44. Driessen, A.J.M., Wickner, W. 1991. *Proc. Natl. Acad. Sci. USA* **88**:2471–2475

44a. Economou, T., Wickner, W.T. 1994. *Cell* (*in press*)

45. Emr, S.D., Hanley-Way, S., Silhavy, T.J. 1981. *Cell* **23**:79–88
46. Gardel, C., Johnson, K., Jacq, A., Beckwith, J. 1990. *EMBO J.* **9**:3209–3216
47. Geller, B.L. 1990. *J. Bacteriol.* **172**:4870–4876
48. Geller, B.L., Green, H.M. 1989. *J. Biol. Chem.* **264**:16465–16469
49. Geller, B.L., Wickner, W. 1985. *J. Biol. Chem.* **260**:13281–13285
50. Geller, B.L., Zhu, H.-Y., Cheng, S., Kuhn, A., Dalbey, R. 1993. *J. Biol. Chem.* **268**:9442–9447
51. Görlich, D., Prehn, S., Hartmann, E., Kalies, K.-U., Rapoport, T.A. 1992. *Cell* **71**:489–503
52. Görlich, D., Rapoport, T.A. 1993. *Cell* **75**:615–630
53. Green Jensen, C., Brown, S., Pedersen, S. 1994. *J. Bacteriol.* **176**:2502–2506
54. Haandrikman, A.J., Kok, J., Soemitro, S., Ledeboer, A.M., Konings, W.N., Venema, G. 1989. *J. Bacteriol.* **171**:2789–2794
55. Hardy, S.J.S., Randall, L.L. 1991. *Science* **251**:439–443
56. Hartl, F.-U., Lecker, S., Schiebel, E., Hendrick, J.P., Wickner, W. 1990. *Cell* **63**:269–279
57. Hartmann, E., Sommer, T., Prehn, S., Görlich, D., Jentsch, S., Rapoport, T.A. 1994. *Nature* **367**:654–657
58. von Heijne, G. 1990. *J. Membrane Biol.* **115**:195–201
59. Hendirck, J.P., Wickner, W. 1991. *J. Biol. Chem.* **266**:24596–24600
60. Hikita, C., Mizushima, S. 1992. *J. Biol. Chem.* **267**:4882–4888
61. Hikita, C., Mizushima, S. 1992. *J. Biol. Chem.* **267**:12375–12379
62. Inouye, M., Halegoua, S. 1980. *CRC Crit. Rev. Biochem.* **7**:339–371
63. Ito, K. 1992. *Mol. Microbiol.* **6**:2423–2428
64. Ito, K., Akiyama, Y. 1991. *Mol. Microbiol.* **5**:2243–2253
65. Jacobs, M., Andersen, J.B., Kontinen, V.P., Sarvas, M. 1993. *Mol. Microbiol.* **8**:957–966
66. Jarosik, G.P., Oliver, D.B. 1991. *J. Bacteriol.* **173**:860–868
67. Johnson, A.E. 1993. *Trends Biochem. Sci.* **18**:456–458
68. Joly, J.C., Wickner, W. 1993. *EMBO J.* **12**:255–263

68a. Joly, J.C., Leonard, M.R., Wickner, W.T. 1994. *Proc. Natl. Acad. Sci. USA* **91**:4703–4707

69. Kamitani, S., Akiyama, Y., Ito, K. 1992. *EMBO J.* **11**:57–62
70. Kato, M., Mizushima, S. 1993. *J. Biol. Chem.* **268**:3586–3593
71. Kato, M., Tokuda, H., Misushima, S. 1992. *J. Biol. Chem.* **267**:413–418
72. Kawasaki, S., Mizushima, S., Tokuda, H. 1993. *J. Biol. Chem.* **268**:8193–8198
73. Kimura, E., Akita, M., Matsuyama, S.I., Mizushima, S. 1991. *J. Biol. Chem.* **266**:6600–6606
74. Klose, M., Schimz, K.-L., van der Wolk, J., Driessen, A.J.M., Freudl, R. 1993. *J. Biol. Chem.* **268**:4504–4510
75. Knott, T.G., Robinson, C. 1994. *J. Biol. Chem.* **269**:7843–7846
76. Kontinen, V.P., Saris, P., Sarvas, M. 1991. *Mol. Microbiol.* **5**:1273–1283
77. Koonin, E.V., Gorbalenya, A.E. 1992. *FEBS Lett.* **298**:6–8
78. Kumamoto, C.A. 1991. *Mol. Microbiol.* **5**:19–22
79. Kumamoto, C.A., Francetic, O. 1992. *J. Bacteriol.* **175**:2184–2188
80. Kumamoto, C.A., Gannon, P.M. 1988. *J. Biol. Chem.* **263**:11554–11558
81. Kusters, R., Breukink, E., Gallusser, A., Kuhn, A., de Kruijff, B. 1994. *J. Biol. Chem.* **269**:1560–1563
82. Kusters, R., Dowham, W., de Kruijff, B. 1991. *J. Biol. Chem.* **266**:8659–8662
83. Kusters, R., Huijbregts, R., de Kruijff, B. 1992. *FEBS Lett.* **308**:97–100
84. Kusukawa, N., Yura, T., Ueguchi, C., Akiyama, Y., Ito, K. 1989. *EMBO J.* **8**:3517–3521
85. Laminet, A.A., Kumamoto, C.A., Plückthun, A. 1991. *Mol. Microbiol.* **5**:117–122
86. Lecker, S., Driessen, A.J.M., Wickner, W. 1990. *EMBO J.* **9**:2309–2314
87. Lecker, S., Lill, R., Ziegelhoffer, T., Bassford, P.J., Jr., Kumamoto, C.A., Wickner, W. 1989. *EMBO J.* **8**:2703–2709
88. Liebke, H.H. 1987. *J. Bacteriol.* **169**:1174–1181
89. Lill, R., Cunningham, K., Brundage, L.A., Ito, K., Oliver, D., Wickner, W. 1989. *EMBO J.* **8**:961–966
90. Lill, R., Dowhan, W., Wickner, W. 1990. *Cell* **60**:271–280
91. Liu, G., Topping, T.B., Randall, L.L. 1989. *Proc. Natl. Acad. Sci. USA* **86**:9213–9217
92. Lu, K., Yamada, H., Mizushima, S. 1991. *J. Biol. Chem.* **266**:9977–9982
93. Luirink, J., Dobberstein, B. 1994. *Mol. Microbiol.* **11**:9–13
94. Luirink, J., ten Hagen-Jongman, C.M., van der Weijden, C.C., Oudega, B., High, S., Dobberstein, B., Kusters, R. 1994. *EMBO J.* **13**:2289–2295
95. Luirink, J., High, S., Wood, H., Giner, A., Tollervey, D., Dobberstein, B. 1992. *Nature* **359**:741–743
96. Martin, J.L., Bardwell, J.C.A., Kuriyan, J. 1993. *Nature* **365**:464–468
97. Matsuyama, S.I., Akimaru, J., Mizushima, S. 1990. *FEBS Lett.* **269**:96–100
98. Matsuyama, S.-I., Fujita, Y., Mizushima, S. 1993. *EMBO J.* **12**:265–270
99. Matsuyama, S.-I., Fujita, Y., Sagara, K., Mizushima, S. 1992. *Biochim. Biophys. Acta* **1122**:77–84

100. Matsuyama, S.I., Kimura, E., Mizushima, S. 1990. *J. Biol. Chem.* **265:**8760–8765
101. Miller, J.D., Bernstein, H.D., Walter, P. 1994. *Nature* **367:**657–659
102. Miller, J.D., Wilhelm, H., Gierasch, L., Gilmore, R., Walter, P. 1993. *Nature* **366:**351–354
103. Missiakes, D., Georgopoulos, C., Raina, S. 1993. *Proc. Natl. Acad. Sci. USA* **90:**7084–7088
104. Missiakes, D., Georgopoulos, C., Raina, S. 1994. *EMBO J.* **13:**2013–2020
105. Mitchell, C., Oliver, D. 1993. *Mol. Microbiol.* **10:**483–497
105a. Mothes, W., Prehn, S., Rapoport, T.A. 1994. *EMBO J.* **13:**3973–3982
106. Murphy, C.K., Beckwith, J. 1994. *Proc. Natl. Acad. Sci. USA* **91:**2557–2561
107. Müsch, A., Wiedmann, M., Rapoport, T.A. 1992. *Cell* **69:**343–352
108. Nishiyama, K.-I., Misuzhima, S., Tokuda, H. 1992. *J. Biol. Chem.* **267:**7170–7176
109. Nishiyama, K.-I., Misuzhima, S., Tokuda, H. 1993. *EMBO J.* **12:**3409–3415
109a. Nishiyama, K.-I., Hanada, M., Tokuda, H. 1994. *EMBO J.* **13:**3272–3277
110. Oliver, D.B., Cabelli, R.J., Dolan, K.M., Jarosik, G.P. 1990. *Proc. Natl. Acad. Sci. USA* **87:**8227–8231
111. Oliver, D.B., Cabelli, R.J., Jarosik, G.P. 1990. *J. Bioenerg. Biomembr.* **22:**311–338
112. Pfanner, N., Neupert, W. 1985. *EMBO J.* **4:**2819–2825
113. Phillips, G.J., Silhavy, T.J., 1990. *Nature* **344:**882–884
114. Phillips, G.J., Silhavy, T.J. 1992. *Nature* **359:**744–746
115. Phoenix, D., Kusters, R., Hikita, C., Mizushima, S., de Kruijff, B. 1993. *J. Biol. Chem.* **268:**17069–17073
116. Pogliano, J.A., Beckwith, J. 1993. *Genetics* **133:**763–773
117. Pogliano, J.A., Beckwith, J. 1994. *EMBO J.* **13:**554–561
118. Pogliano, J.A., Beckwith, J. 1994. *J. Bacteriol.* **176:**804–814
119. Poritz, M.A., Bernstein, H.D., Strub, K., Zopf, D., Wilhelm, H., Walter, P. 1990. *Science* **250:**1111–1117
120. Poritz, M.A., Strub, K., Walter, P. 1988. *Cell* **55:**4–6
121. Pugsley, A. 1993. *Microbiol. Rev.* **57:**50–108
122. Ulbrandt, N.D., London, E., Oliver, D.B. 1992. *J. Biol. Chem.* **267:**15148–15192
123. Randall, L.L. 1983. *Cell* **33:**231–240
124. Randall, L.L. 1992. *Science* **257:**241–245
125. Randall, L.L., Hardy, S.J.S. 1986. *Cell* **46:**921–928
126. Randall, L.L., Topping, T.B., Hardy, S.J.S. 1990. *Science* **248:**860–864
127. Rapoport, T.A. 1992. *Science* **258:**931–936
128. Ribes, V., Römisch, K., Giner, A., Dobberstein, B., Tollervey, D. 1990. *Cell* **63:**591–600
129. Römisch, K., Webb, J., Herz, J., Prehn, S., Frank, R., Vingron, M., Dobberstein, B. 1989. *Nature* **340:**478–482
130. Saier, M.H. 1994. *FEBS Lett.* **337:**14–17
131. Sanders, S.L., Whitfield, K.M., Vogel, J.P., Rose, M.D., Schekman, R.W. 1992. *Cell* **69:**353–365
132. Schatz, P.J., Beckwith, J. 1990. *Annu. Rev. Genet.* **24:**215–248
133. Schatz, P.J., Bieker, K.L., Ottemann, K.M., Silhavy, T.J., Beckwith, J. 1991. *EMBO J.* **10:**1749–1757
134. Schatz, P.J., Riggs, P.D., Jacq, A., Fath, M.J., Beckwith, J. 1989. *Genes Dev.* **3:**1035–1044
135. Shevchik, V.E., Condemine, G., Robert-Baudouy, J. 1994. *EMBO J.* **13:**2007–2012
136. Schiebel, E., Driessen, A.J.M., Hartl, F.-U., Wickner, W. 1991. *Cell* **64:**927–939
137. Schiebel, E., Wickner, W. 1992. *J. Biol. Chem.* **267:**7505–7510
138. Schmid, M.G., Dolan, K.M., Oliver, D. 1991. *J. Bacteriol.* **173:**6605–6611
139. Shimoike, T., Akiyama, Y., Baba, T., Taura, T., Ito, K. 1992. *Mol. Microbiol.* **6:**1205–1210
140. Shinkai, A., Mei, L.H., Tokuda, H., Mizushima, S. 1991. *J. Biol. Chem.* **266:**5827–5833
141. Shiozuka, K., Tani, M., Mizushima, S., Tokuda, H. 1990. *J. Biol. Chem.* **31:**18843–18847
142. Simon, S.M., Blobel, G. 1991. *Cell* **65:**371–380
143. Simon, S.M., Blobel, G. 1992. *Cell* **69:**677–684
144. Simon, S.M., Peskin, C.S., Oster, G.F. 1992. *Proc. Natl. Acad. Sci. USA* **89:**3770–3774
145. Simonen, M., Palva, I. 1993. *Microbiol. Rev.* **57:**109–137
146. Swidersky, U.E., Rienhöfer-Schweer, Werner, P.K., Ernst, F., Benson, S.A., Hoffschulte, H.K., Müller, M. 1992. *Eur. J. Biochem.* **207:**803–811
147. Tani, K., Mizushima, S. 1991. *FEBS Lett.* **285:**127–131
148. Tani, K., Shiozuka, K., Tokuda, H., Mizushima, S. 1989. *J. Biol. Chem.* **264:**18582–18588
149. Tani, K., Tokuda, H., Mizushima, S. 1990. *J. Biol. Chem.* **265:**17341–17347
150. Taura, T., Baba, T., Akiyama, Y., Ito, K. 1993. *J. Bacteriol.* **175:**7771–7775
151. Tokuda, H., Akimaru, J., Matsuyama, S.I., Nishuyama, K., Mizushima, S. 1991. *FEBS Lett.* **279:**233–236
152. Tokuda, H., Kim, Y.L., Mizushima, S. 1990. *FEBS Lett.* **264:**10–12
153. Tomassen, J., Filloux, A., Bally, M., Murgier, M., Lazdunski, A. 1992. *FEMS Microbiol. Rev.* **103:**73–90
154. Van der Goot, F., Didat, N., Pattus, F., Dowham, W., Letellier, L. 1993. *Eur. J. Biochem.* **213:**217–221
155. de Vrije, T., de Swart, R.L., Dowham, W., Tommassen, J., de Kruijff, B. 1988. *Nature* **334:**173–175
156. Walker, J.E., Saraste, M., Runswick, M.J., Gay, N.J. 1982. *EMBO J.* **1:**945–951
157. Watanabe, M., Blobel, G. 1989. *Cell* **58:**695–705
158. Watanabe, M., Blobel, G. 1989. *Proc. Natl. Acad. Sci. USA* **86:**1895–1899
159. Watanabe, M., Blobel, G. 1993. *Proc. Natl. Acad. Sci. USA* **90:**9011–9015
160. Watanabe, M., Nicchitta, C.V., Blobel, G. 1990. *Proc. Natl. Acad. Sci. USA* **87:**1960–1964
161. Weiss, J.P., Ray, P.H., Bassford, P.J., Jr. 1988. *Proc. Natl. Acad. Sci. USA* **85:**8978–8982
162. Wickner, W., Driessen, A.J.M., Hartl, F.-U. 1991. *Annu. Rev. Biochem.* **60:**101–124
163. Wunderlich, M., Otto, A., Seckler, R., Glockshuber, R. 1993. *Biochemistry* **32:**12251–12256
164. van der Wolk, J., Klose, M., Breukink, E., Demel, R.A., de Kruijff, B., Freudl, R., Driessen, A.J.M. 1993. *Mol. Microbiol.* **8:**31–42
165. van der Wolk, J., Klose, M., Freudl, R., Driessen, A.J.M. 1994. Preprotein binding by ATP-binding site mutants of the bacillus subtilis Sec A. *In:* Biological Membranes: Structure, Biogenesis and Dynamics. J.A.F. Op den Kamp, editor. NATO ASI Series. Vol. H 82, pp. 237–244. Springer-Verlag, Heidelberg
166. Yamada, H., Matsuyama, S.-I., Tokuda, H., Mizushima, S. 1989. *J. Biol. Chem.* **264:**18577–18581
167. Yamada, H., Tokuda, H., Mizushima, S. 1989. *J. Biol. Chem.* **264:**1723–1728
168. Zimmermann, R., Watts, C., Wickner, W. 1982. *J. Biol. Chem.* **257:**6529–6536